Tetrazolium Compounds. Part III. Styryl Derivatives.*

By A. W. NINEHAM, D. L. PAIN, and R. SLACK.

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The preparation of a number of 1 : 3-disubstituted 5-styrylphenylformazans † and of the corresponding tetrazolium salts is described.

PART II * of this series described the preparation and properties of tetrazolium salts in which benzene rings attached to the tetrazolium nucleus carried phenylazo-substituents. The present paper describes a similar series of salts in which the attached benzene rings bear styryl, or in a few cases, both styryl and phenylazo-groups. The compounds were prepared in order to examine the effect of extended conjugation on the colour of the formazans and on the biological activity of the tetrazolium salts. 2 : 5-Diphenyl-3-*p*-styrylphenyltetrazolium chloride has been used by Henley (Ann. Report, Dept. Rheumatic Diseases, W. London Hospital, 1952, p. 24) for the colorimetric estimation of small amounts of cortisone.

Most of the intermediates required are known substances. 4-Amino-4'-hydroxy- (Brownlee, Copp, Duffin, and Tonkin, *Biochem. J.*, 1943, **37**, 572) and 4-acetamido-4'-aminostilbene (Brode and Piper, *J. Amer. Chem. Soc.*, 1941, **63**, 1502) were prepared by

* Part II, preceding paper. † See Part I, *J.*, 1953, 3881, for nomenclature of formazans.

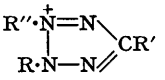
iron-dust reductions of the corresponding nitro-compounds and Cullinane's method (*J.*, 1923, **123**, 2056) was applied to the preparation of 4-bromo-4'-nitrostilbene (l'Ecuyer, Giguère, Olivier, and Roberge, *Canad. J. Res.*, 1948, **26**, *B*, 70). 4-Amino-4'-bromostilbene (mentioned but not described by Thompson, Vago, Corfield, and Orr, *J.*, 1950, 214) was obtained from this by reduction with stannous chloride.

TABLE 1. *Formazans*, R·NH·N:CR'·N:NR''.

	R	R'	R''	Yield (%)	M. p.	Appearance	Solvent
1	Ph	Me	*p*-C_6H_4·CH:CHPh	68	160—162°	Dark orange [a]	A
2	Ph	Me	*p*-C_6H_4·CH:CH·C_6H_4·NO_2-*p*	16	182—183	Dark orange [a]	B
3	Ph	Ph	*p*-C_6H_4·CH:CHPh	74	185	Purple, green reflex [b]	C
4	Ph	*p*-C_6H_4·OMe	,,	83	157—158	Purple [a]	D
5	Ph	*p*-C_6H_4Br	,,	47	170—171	Purple [b]	D
6	Ph	Ph	*p*-C_6H_4·CH:CH·C_6H_4·NO_2-*p*	33	185—186	Purple, golden reflex [b]	E
7	Ph	Ph	*p*-C_6H_4·CH:CH·C_6H_4Br-*p*	33	186—187	Purple [c]	E
8	Ph	Ph	*p*-C_6H_4·CH:CH·C_6H_4·OH-*p*	32	175—176	Purple [d]	B
9	Ph	Ph	*p*-C_6H_4·CH:CH·C_6H_4·NHAc-*p*	14	208—209	Purple, green reflex [b]	B
10	*p*-C_6H_4·N:NPh	Ph	*p*-C_6H_4·CH:CHPh	47	*	Purplish black, red reflex	D
11	,,	*p*-C_6H_4·CO_2H	,,	33	239—240	Black	F
12	,,	*p*-C_6H_4·OAc	,,	40	220—221	Purple [d]	E
13	Ph	Me	*p*-C_6H_4·CH:CH·C_6H_4·NHAc-*p*	12	203	Red [d]	G

A, Ethanol; B, nitromethane; C, ethyl acetate; *D*, *cyclo*hexane; *E*, benzene; F, acetone; G, nitroethane.

* Not obtained analytically pure. [a] Plates. [b] Needles. [c] Rods. [d] Prisms.

TABLE 2. *Tetrazolium salts*, 

No.*	Anion	Method of oxidn.	Yield (%)	M. p.	Appearance	Solvent
1	I	(i)	34	169—172°	Yellow needles	A
2	I	(i)	28	222—223	Yellow	B
3a	Cl	(i)	54	228	Yellow needles	C
3b	Isethionate	—	37	181	Yellow prisms	B
3c	$\frac{1}{2}SO_4$	—	86	†	Yellow	B
3d	HSO_4	—	71	169—171	Yellow needles	D
4	I	(i)	39	167—168 †	,,	E
5	I	(i)	14	206 †	,,	F
6	Cl	(i)	57	233—234	Pale yellow prisms	B
7	Cl	(i)	67	216—217	Yellow needles	B
8	I	(i)	25	272 †	Orange rods	A
9	I	(i)	41	244 †	Orange rectangular plates	E
10	I	(i)	20	175 † (sinters 151—152)	Orange-red	E
11	Cl	(ii)	11	195 †	Red	E
12	Cl	(ii)	58	215—216 †	Red rods	G

* The tetrazolium salts have the R, R', and R'' of the formazans of corresponding number in Table 1. † With decomp. (i) Mercuric oxide; (ii) *iso*amyl nitrite. Solvents: A, methanol; B, ethanol–ether; C, water; D, 0·5N-sulphuric acid; E, ethanol; F, acetone–methanol–light petroleum (b. p. 60—80°); G, acetone–ether, containing a trace of methanol.

The formazans (see Tables 1 and 3) were prepared by methods already described in Parts I and II. Except for 5-*p*-(4-acetamidostyryl)phenyl-3-methyl-1-phenylformazan, all the formazans were satisfactorily oxidised to tetrazolium salts (see Tables 2 and 4) by standard methods.

Experimental

4-Amino-4'-hydroxystilbene.—4-Hydroxy-4'-nitrostilbene (Cullinane, *loc. cit.*) (11·0 g.) and iron pin-dust (11·0 g.) in Cellosolve (2-ethoxyethanol) (120 c.c.) were heated to 90°, concentrated hydrochloric acid (6 c.c.) in water (3 c.c.) was added slowly, and the suspension stirred vigorously

Table 3. *Analyses of formazans.*

		Found (%)			Required (%)		
No.*	Formula	C	H	N	C	H	N
1	$C_{22}H_{20}N_4$	—	—	16·3	—	—	16·5
2	$C_{22}H_{19}O_2N_5$	68·4	5·0	17·7	68·5	4·9	18·2
3	$C_{27}H_{22}N_4$	80·4	5·8	13·6	80·6	5·5	13·9
4	$C_{26}H_{24}ON_4$	77·7	5·7	—	77·8	5·6	—
5	$C_{27}H_{21}N_4Br$	—	—	11·6	—	—	11·6
6	$C_{27}H_{21}O_2N_5$	72·4	4·9	—	72·4	4·7	—
7	$C_{27}H_{21}N_4Br$	—	—	11·6	—	—	11·6
8	$C_{27}H_{23}ON_4,\frac{1}{2}H_2O$	76·5	5·5	12·9	75·9	5·4	13·1
9	$C_{29}H_{25}ON_5$	75·8	5·5	15·4	75·7	5·5	15·2
11	$C_{34}H_{26}O_2N_6$	74·3	5·0	—	74·2	4·8	—
12	$C_{35}H_{28}O_2N_6$	74·4	5·1	14·5	74·5	5·0	14·9
13	$C_{24}H_{23}ON_5$	72·2	6·0	17·3	72·5	5·8	17·6

* Cf. Table 1.

Table 4. *Analyses of tetrazolium salts.*

		Found (%)					Required (%)				
No.*	Formula	C	H	N	Hal.	S	C	H	N	Hal.	S
1	$C_{22}H_{19}N_4I,\frac{1}{2}H_2O$	—	—	11·8	26·6	—	—	—	11·8	26·7	—
2	$C_{22}H_{18}O_2N_5I$	—	—	13·7	24·6	—	—	—	13·7	24·8	—
3a	$C_{27}H_{21}N_4Cl,H_2O$	—	—	12·3	7·9	—	—	—	12·3	7·8	—
3b	$C_{29}H_{26}O_4N_4S$	—	—	10·9	—	6·2	—	—	10·7	—	6·1
3c	$C_{54}H_{42}O_4N_8S,2H_2O$	—	—	11·9	—	3·4	—	—	12·0	—	3·4
3d	$C_{27}H_{22}O_4N_4S$	—	—	11·9	—	6·5	—	—	11·2	—	6·4
4	$C_{26}H_{23}ON_4I$	—	—	10·1	22·8	—	—	—	10·0	22·8	—
5	$C_{27}H_{20}N_4BrI$	—	—	9·4	70·9 †	—	—	—	9·2	69·8 †	—
6	$C_{27}H_{20}O_2N_5Cl,2H_2O$	—	—	13·5	6·7	—	—	—	13·5	6·8	—
7	$C_{27}H_{20}N_4ClBr,H_2O$	—	—	10·4	63·0 †	—	—	—	10·5	62·1 †	—
8	$C_{27}H_{21}ON_4I$	—	—	10·6	23·3	—	—	—	10·3	23·4	—
9	$C_{29}H_{24}ON_5I,H_2O$	58·0	4·2	11·4	21·7	—	57·7	4·3	11·6	21·1	—
10	$C_{33}H_{25}N_6I$	—	—	12·7	20·7	—	—	—	13·3	20·1	—
11	$C_{34}H_{25}O_2N_6Cl,H_2O$	67·5	4·3	—	6·3	—	67·8	4·5	—	5·9	—
12	$C_{33}H_{25}ON_6Cl,4H_2O$	63·5	4·3	13·4	—	—	63·0	5·2	13·4	—	—

* Cf. Table 2. † Total silver halide.

for 4 hr. The mixture was cooled, neutralised with sodium carbonate (6 g.), and diluted with water to give the amine (62%).

4-Amino-4'-bromostilbene.—4-Bromo-4'-nitrostilbene was reduced with stannous chloride and hydrogen chloride in acetic acid. The stannichloride was decomposed with aqueous 50% sodium hydroxide, and the crude product was extracted from the residue with acetone and crystallised from *sec.*-butanol, to give the *amino-stilbene* (65%) as orange plates, m. p. 203—205° (Found : N, 5·3; Br, 29·0. $C_{14}H_{12}NBr$ requires N, 5·1; Br, 29·2%).

4-Acetamido-4'-aminostilbene.—4-Acetamido-4'-nitrostilbene (Ashley *et al.*, *J.*, 1942, 103) was reduced with iron dust in boiling 90% acetic acid, to give the aminostilbene (92%), m. p. 235° (from anisole).

Preparation of 4-Phenylazophenylhydrazones.—Aldehydes were condensed with *N*-4-phenylazophenylhydrazine-*N'*-sulphonic acid by Träger, Berlin, and Franke's method (*Arch. Pharm.*, **1906**, **244**, 307, 326), to give p-*acetamido*-, brick-red prisms (from ethanol), m. p. 190—191° (Found : C, 67·7; H, 5·8; N, 18·4. $C_{21}H_{19}ON_5,H_2O$ requires C, 67·2; H, 5·7; N, 18·6%), p-*hydroxy*-, lustrous orange plates (from benzene), m. p. 208—209° (Found : C, 73·1; H, 5·1; N, 17·3. $C_{19}H_{16}ON_4$ requires C, 72·1; H, 5·1; N, 17·7%), p-*acetoxy*-, small orange prisms (from ethanol), m. p. 161—162° (Found : N, 15·6. $C_{21}H_{18}O_2N_4$ requires N, 15·6%), and the *ammonium* salt of p-*carboxybenzylidene-4-phenylazophenylhydrazine*, yellow powder (from ethanol), m. p. 245° (Found : C, 66·3; H, 5·0; N, 19·0. $C_{20}H_{19}O_2N_5$ requires C, 66·5; H, 5·3; N, 19·4%).

Tetrazolium Salts.—Tetrazolium chlorides were converted into the sulphates by treatment

with the calculated amount of silver sulphate in boiling water. Addition of dilute sulphuric acid gave the corresponding hydrogen sulphates. The isethionate was obtained by passing a solution of the chloride in aqueous ethanol through a column of " Amberlite IRA 400 (OH^-) " ion-exchange resin and neutralising the filtrate with isethionic acid.

The following could not be isolated : 5-*p*-(4-bromostyryl)phenyl-3-methyl-1-phenyl-, 5-*p*-(4-hydroxystyryl)phenyl-3-methyl-1-phenyl-, 3-(2 : 4-dichlorophenyl)-1-phenyl-5-*p*-styrylphenyl-, 1-phenyl-3-styryl-5-*p*-styrylphenyl-, 3-*p*-hydroxyphenyl-1-*p*-phenylazophenyl-5-*p*-styrylphenyl- and 3-*p*-acetamidophenyl-1-*p*-phenylazophenyl-5-*p*-styrylphenyl-formazan.

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